

STUDIES ON IMMUNOMODULATORY ACTIVITY of *Aloe vera* (Linn)

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ABSTRACT: To study the immunomodulatory activity of saline extracts of leaves of *Aloe vera* Linn. (Family: Liliaceae) on the albino mice. The saline extract of leaves of *Aloe vera* was administered orally according to their body weight in mice. The assessment of immunomodulatory activity on specific and nonspecific immunity was studied by administration of test extract. The method of pyrogallol induced immunosuppression was employed with slight modification to study the immunomodulatory potential of the extract. *Humoral antibody response to SRBC* measurement of antibody titer by haemagglutination reaction was done and cellular immune response (Foot pad reaction test) the edema was induced in the right paw of mice by injecting SRBC (0.025x10⁹ cells) in the sub planar region. Pyrogallol-induced suppression of humoral as well as cell mediated immune response was significantly attenuated by daily oral treatment with saline extract of *Aloe vera*. Vitamin E treated group exhibited similar attenuation of the suppression in immune responses. *Aloe vera* extract at the dose of 100 mg/kg was found to suppress delayed type hypersensitivity reaction induced by SRBCs in mice. As evidenced by marked increase in haemagglutination titers in mice was also observed. The study demonstrates that *A. vera* triggers both specific and non-specific responses to a greater extent. The study comprised the acute toxicity and preliminary phytochemical screening of *A. vera*. From the results obtained and phytochemical studies the immunostimulant effect of *Aloe vera* could be attributed to the alkaloids content.

Keywords: Immunomodulatory activity, *Aloe vera*, Haematological, Serological studies.

INTRODUCTION

The natural resistance of the body against infection can be enhanced by the use of herbal drugs.¹ several herbal preparations that can enhance the body's immune system status are extensively being used in the indigenous system of medicines. There is an upsurge in the clinical usage of indigenous drugs as they are free from serious side effects. Dua *et al.*² reported a large number of plants having a known immunomodulatory activity. The interactions of large molecules in biological systems play an important part in many life processes. Both polysaccharides and glycoprotein are involved in such activities, especially in connection with the immune system. The immune system itself is very much more complex, having at its centre the reaction of a host's antibodies with invasive antigens³. *Aloe vera* one of the most widely used healing plants in the history of mankind⁴. *Aloe vera* is one of the few substances known to effectively decrease inflammation and promote wound healing⁵. It has also been investigated for its antioxidant property⁶. Therefore the present study was conducted to investigate the immunomodulatory activity of *Aloe vera* extract.

MATERIALS AND METHODS

Plant material

Specimen of *Aloe Vera* (L) was harvested from Tindal, Erode district, Tamil Nadu and collected plant has been identified by Botanical survey of India at Coimbatore, fresh leaves of this cultivated plant were used in this study.

Extraction

Aloe vera leaves (6 big and healthy leaves) weighed, washed and cut in the middle, the gel were separated by scratching with a spoon and pulp was cut in to the small pieces (514 gms) and homogenized with phosphate buffered saline solution (PBS; pH 7; 600 ml) by means of a blender. The extract was kept at 4°C overnight, and then filtered through muslin cloth and the filtrate. Centrifuged at 20000 rpm for 30 mins at 2°C in a refrigerated centrifuged. The green pallet was discarded and the clear yellow supernatant was taken and lyophilized, thus 10 gms of *Aloe vera* leaves pulp extract was obtained .the extract used in the experiment was (7.5%) prepared by dissolving the powder in Phosphate buffer solution and mixing it thoroughly via magnetic stirrer⁷.

Experimental protocols

Healthy mice (25-30 gms) of either sex were selected for the study. The animals were fed on a commercial diet water and libitum (Hindustan lever pellets, Bangalore). They were acclimated to laboratory hygienic conditions for ten days before starting the experiment. Permission of the Institutional animal ethical committee was obtained for all animal experimentation as per the approved protocol (NCP/IAEC/PG/04/2009)⁸.

Administration of test extract:

The method of pyrogallol induced immunosuppression was employed with slight modification to study the immunomodulatory potential of the extract. Animals were randomly divided into four groups, consisting of six animals each. Group I animals served as control and received equivalent volume of sodium CMC (0.1 % w/v) as a vehicle. Group II animals were administered pyrogallol (100 mg/kg *i.p.* daily for seven days). Group III animals were given pyrogallol daily for seven days with the same dose and the test extract, suspended in 0.1 % (w/v) sodium carboxymethyl cellulose (CMC) with 100 mg/kg daily, *p.o.* from day 8 to 22. Group IV animals were given with pyrogallol daily for 7 days and vitamin E suspension (150 mg/kg *p.o.*) beside above treatment, all the groups received sheep red blood cells (SRBC, 0.5 x 10⁹ cells/100 g, *i.p.*) on day 7 and 13, as the antigenic material to sensitize them for immunological studies⁸.

Humoral antibody response to SRBC

Measurement of antibody titer by haemagglutination reaction was done by the method of Miller *et al* On 13th and 20th day, blood samples were collected from the retro-orbital plexus and the mice serum was used for determination of haemagglutination titer. The blood samples were centrifuged to collect serum and equal volume of individual serum samples of each group was pooled. To serial two fold dilutions of pooled serum samples made in 25 µl in normal saline in micro titration plates was added 25 µl of 1% SRBC suspension in saline. After mixing, the plates were incubated at 37°C for 1 hrs and examined for haemagglutination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titer.

Cellular immune response (Foot pad reaction test)

To study the cellular immune response, the edema was induced in the right paw of mice by injecting SRBC (0.025x10⁹ cells) in the sub planar region on 20th day, the increase in paw volume in 48 hrs, i.e. on 22nd day was assessed on digital Plethysmometer (UGO basile- 7150) The mean percentage increase in foot pad volume was considered as delayed type hypersensitivity and as an index of cell mediated immunity. The volume of the left hind paw, injected similarly with phosphate buffered saline served as a control.

RESULTS AND DISCUSSION

Pyrogallol-induced suppression of humoral as well as cell mediated immune response were significantly attenuated by daily oral treatment with *Aloe Vera* extract. Vitamin E treated group exhibited similar attenuation of the suppression in immune responses. *A. vera* extract at the dose of 100 mg/kg was found to suppress delayed type hypersensitivity reaction induced by SRBCs in mice (**Table 2**). It reveals effect of drug on T-lymphocytes and other cell types required for expression of humoral response to SRBCs, as evidenced by marked increase in haemagglutination titers in mice was also observed (**Table 1**). In conclusion, the results obtained in the present study showed that *A. vera* extract produces stimulatory effect on the humoral and cell mediated immune response in the experimental animals and suggest its therapeutic usefulness in disorders of immunological origin. Further studies using *in-vivo* and *in-vitro* models of immunomodulation are needed to confirm the immunomodulatory activity of plant *Aloe vera* and its mechanism of action

Table 1: Influence of *A.vera* extract and vitamin E on the primary and secondary humoral immune responses to sheep RBC.

S. No.	Group	Antibody titer of primary	Antibody titer of secondary
1.	Control group normal (saline)	7.6±0.22	10.3 ±0.18
2.	Immunosuppressed(Pyrogallol treated) group	5.2±0.16	7.22±0.20
3.	Experimental (100 mg/kg alcoholic extract of <i>F.religiosa</i>)	5.8 ± 0.26	6.38±0.24
4.	Standard (Vitamin E suspension, 150.0 mg/kg)	7.33±0.51	10.66±0.81

(n=6) ± Standard deviation, p < 0.05, when compared with control group in all cases (Statistics; One way ANOVA Followed by Dunnet's test)

Table 2: Influence of *A. vera* extract and vitamin E on cell mediated immune responses to Sheep RBC.

S. No.	Groups	Groups Mean increase in paw volume
1.	Control group (Normal saline)	29.36±0.35
2.	Immunosuppressed (Pyrogallol treated) group	15.28±0.38
3.	Experimental(100 mg/kg extract of aloe vera)	19.37±0.46
4.	Standard(Vitamin E suspension 150.0 mg/kg)	21.73±2.47

(n=6) ± Standard deviation, p < 0.05, when compared with control group in all cases (Statistics; One way ANOVA Followed by Dunnet's test)

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